REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE -	3. REPORT TYPE AND DATES COVERED FINAL 09/01/97 - 08/31/01

4. TITLE AND SUBTITLE
Biochemically Vunreable Sites for Antifungal Intercession in the Control of Fungal Growth

5. FUNDING NUMBERS
DAAH04-93-D-0003

6. AUTHOR(S)
Leo Parks

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
North Carolina State University

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U. S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
37372.8-LS-SR

11. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12 a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12 b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

In the present grant period DNA array experiments were done in an approach towards identifying target genes that might be transcriptionally regulated by *UPC2*. Nylon membrane supported, genomic scale arrays were hybridized with radiolabeled cDNA from various strains, which were wild type or mutant with various alleles of *UPC2*. *PDA1* was chosen to normalize the data. While many candidate genes were identified, expression of the genes *ERG2, ERG2, ERG11,* and *ERG25* were selected for additional study. No difference from wild-type levels of *ERG2* and *ERG3* was apparent except in the *upc2-1* mutant.

14. SUBJECT TERMS	15. NUMBER OF PAGES 2
	16. PRICE CODE

17. SECURITY CLASSIFICATION OR REPORT	18. SECURITY CLASSIFICATION ON THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev.2-89)
Prescribed by ANSI Std. 239-18
298-102

20020125 291

Summary Progress Report

Effects on transcriptional regulation of ergosterol biosynthesis by *UPC2*, a gene involved in the control of sterol uptake in the yeast, *Saccharomyces cerevisiae*, have been investigated in this project. While an enormous expenditure of energy is required to produce ergosterol, wild-type yeast strains grown in aerobic conditions will not take up appreciable amounts of sterol from the growth media. However, under anaerobic conditions where sterol synthesis is precluded by the absence of oxygen, uptake of sterol is required for viability. This seeming paradox, termed aerobic sterol exclusion, is breached in the *UPC2* mutant strains. Evidence consistent with a regulatory role for *UPC2* has been gathered from several studies. Firstly, the *upc2-1* phenotype was shown in our previous work to be pleiotropic, resulting in cation sensitivity, increased sterol esterification that was independent of growth phase, and an enhancement of sterol synthesis. Secondly, a mull mutation of *UPC2* conferred neither the osmotic hypersensitivity nor the increased sterol uptake that was observed in the point mutations. Finally, the predicted sequence for the Upc2p contains the Zn(II)2Cys6 cluster DNA binding motif that is affiliated with a group of fungal regulatory proteins. Our previous results from northern blots indicated that several late ergosterol biosynthetic genes are up-regulated in a *upc2-1* background.

In the present grant period DNA array experiments were done in an approach towards identifying target genes that might be transcriptionally regulated by *UPC2*. Nylon membrane supported, genomic scale arrays were hybridized with radiolabeled cDNA from various strains, which were wild type or mutant with various alleles of *UPC2*. *PDA1* was chosen to normalize the data. While many candidate genes were identified, expression of the genes *ERG2, ERG2, ERG11,* and *ERG25* were selected for additional study. No difference from wild-type levels of *ERG2* and *ERG3* was apparent except in the *upc2-1* mutant.

Semi-quantitative RT-PCR was used to obtain further evidence and confirmation of differential expression for the later genes involved in ergosterol biosynthesis. This approach allows for the detection and relative measurement of RNA molecules present even at extremely low levels in the cell. The four ERG genes identified above were tested by this method. The most dramatic results were with the *upc2-1* point mutant. In that strain 0.6 to 2.4 fold increases were observed.

As a final test of the hypothesis that *UPC2* affects transcriptional regulation of *ERG3,* galactosidase assays were done for strains with pERG3-lacZ congenic fusion constructs. Again the *upc2-1* mutant strain demonstrated a 3-fold increase in specific activity for the reporter gene.

Based on the combined methods of this study, we have concluded that *ERG2, ERG3, ERG11,* and *ERG25* mRNA levels are increased as a specific result of the *upc2-1* point mutation. While minor increases in the expression of some of these genes were associated with the *upc2* null allele, these levels are essentially unchanged from that resulting from the wild-type *UPC2* allele. Induction of some of the genes of ergosterol

biosynthesis has been shown by others to result from growth of the organisms with the sterol-lowering agent, lovastatin. This was shown to require the Upc2p protein.

Students: Two graduate students were involved with this project, Kevin Shianna and W. David Dotson. Both received their Ph.D. degrees and are now involved in Post-Doc positions. Dr. Leo Parks was in charge of the lab and Dr. Sherry Tove was involved as part of an ARO staff research project. Five papers were published during the three years of this research.